# Nitrogen Fixation:
## Achievements and Objectives

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# Identification of Bacteriohopanetetrol in *Frankia*

Cytological evidence indicates that high levels of lipids are associated with nitrogen-fixing tissue in actinorhizal root nodules (1). The purpose of the investigation was to characterize the lipid profile of actinorhizal nodules using a new method to separate lipid classes (2), and then to identify resulting compounds of interest.

Lipid-containing extracts were prepared by conventional methods from root nodules of two hosts, *Alnus rubra* and *Ceanothus griseus* var. *horizontalis*. Extracts were prepared from root tips of the hosts, and from two strains of *Frankia*, HFPArI3 and HFPCcI3. Lipids classes were separated and quantified by a new HPLC method which employs a flame ionization detector (2).

An unusual, major lipid peak was detected in nodule tissue extracts, representing 20-30% of total extracted nodule lipids. The lipid was also present in *Frankia* extracts, but not in host root extracts, or in a plant lipid standard mixture. The unknown lipid was identified by mass spectrometry (with acetylation) to be bacteriohopanetetrol. The molecular ion of the tetraacetate was 714, and major ion fragments had m/z of 191, 369, 493 and 654. In *Frankia*, bacteriohopanetetrol represented 30-50% of total extractable lipids, in ammonium-grown as well as in $N_2$-grown cells.

Bacteriohopanetetrol ($C_{35}H_{62}O_4$) is a hopanoid, or pentacyclic triterpenoid, with a hydroxylated side chain. Although this is the first report of the occurrence of bacteriohopanetetrol in *Frankia*, hopanoids have been shown to occur in numerous bacterial species (3). This amphiphilic, sterol-like molecule probably functions in stabilizing *Frankia* membranes, and could play a role in maintaining functional nitrogenase activity in the *Frankia* vesicle.

REFERENCES


1. Berry, A.M., Sunell, L.A. & Liu. Q. (1989) in *Physiology, Biochemistry and Genetics of Nongreen Plastids*, eds. Boyer, C., Shannon, J., & Hardison, R. (A.S.P.P., Rockville, MD), 287.
2. Moreau, R.A., Asmann, P.T. & Norman, H.A. (in press) Phytochemistry.
3. Rohmer, M., Bouvier-Navé, P. & Ourisson G. (1984) J. Gen. Microbiol. **130**, 1137-1150.

# Identification of Bacteriohopanetetrol in *Frankia*

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